Ionic Liquid-Modified Silica as Solid Phase Extraction Material Coupled with Spectrofluorimetry for the Analysis of Honokiol and Magnolol in Traditional Chinese Medicine

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Abstract

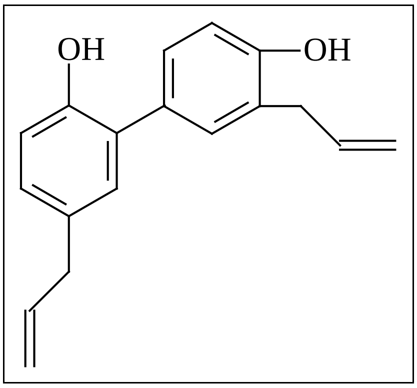
Novel ionic liquid-modified silica particles (IL-SiO_2) were prepared by the surface chemical modification of the commercial silica. The obtained ionic liquid-modified particles were successfully used as solid-phase extraction (SPE) material coupled to fluorescence spectroscopy for the determination of honokiol and magnolol which are active constituents of traditional Chinese Medicine. Several factors influencing the pre-concentration/separation of honokiol and magnolol were discussed in details. Under the optimal conditions, the adsorption efficiencies of honokiol and magnolol with IL-SiO_2 as sorbents were much higher than those with bare silica (IL-SiO_2: 97.2%, 96.6%; SiO_2: 17.6%, 2.5%). The adsorption capacities of IL-SiO_2 for honokiol and magnolol were 6.9 $mg \cdot g^{-1}$ and 6.0 $mg \cdot g^{-1}$. The linear range of calibration curve for the determination of honokiol and magnolol was 0.015-7.60, 0.008-5.00 $\mu g \cdot mL^{-1}$, and the limit of detection (LOD) was 2.6, 1.3 $ng \cdot mL^{-1}$, respectively, with enrichment factor of 6. The developed method was successfully applied to the determination of honokiol and magnolol in Huoxiang Zhengqi Shui.

Keywords

Ionic Liquid-Modified Silica; Honokiol and Magnolol; Solid-Phase Extraction; Spectrofluorimetry

Introduction

Honokiol and magnolol are the main constituents simultaneously identified in the barks of Magnolia officinalis (Chinese name: Houpo) which have been used in traditional Chinese medicine to treat a variety of mental disorders [Bang et al, 2000]. Many studies have demonstrated that honokiol and magnolol (Fig. 1) have widely pharmacological effects such as anti-inflammatory, antibacterial, anti-tumor, anti-diabetic complications, anti-microbial, anti-neurodegeneration, anti-depressant and so on [Kouyun et al, 2006; Li qing et al, 2005].



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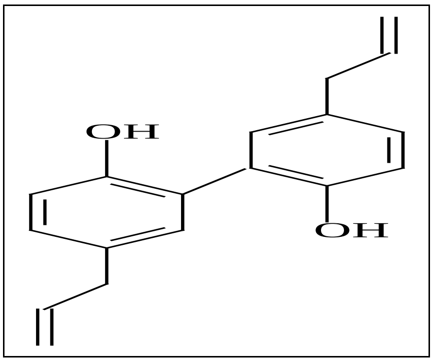

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FIGURE 1 STRUCTURE OF HONOKIOL (A) AND MAGNOLOL (B)

The contents of honokiol and magnolol can be recognized as the quality control standard of Houpo by Pharmacopoeia of People's Republic of China [National Pharmacopoeia Commission, 2005]. Several

methods for the determination of honokiol and magnolol have been described in literatures, such as high-performance liquid chromatography (HPLC) [Xin'an et al, 2003], counter-current chromatography [Chen L. et al, 2007], capillary electrophoresis Chen G. et al, 2006], TLC scanning [Huiping, 2000] and electro-chemical sensor [Jun et al, 2009]. However, methods mentioned above usually are complex and time-consuming while spectrometry is comparatively more simple and sensitive. As known, there are only a few papers reported to determine honokiol and magnolol with spectrometry [Zhi et al, 2004]. Furthermore, the concentrations of honokiol and magnolol in samples (traditional Chinese medicine/blood) are very low and matrix is complex. Therefore, an efficient preconcentration/ separation technique is frequently required.

The solid-phase extraction (SPE) technique is widely used in pre-concentration/separation of trace mineral elements [Marahel et al, 2009], organic compounds [Changlong et al, 2010] and biological molecules [Puoci et al, 2008]. The significant advantages of SPE are high enrichment factors, high recoveries, rapid phase separation, low consumption of organic solvents, and capability to combine with different detection techniques in the form of on-line or off-line mode [Fritz, 1999]. More recently, SPE also offers broader range of applications due to the choice of different sorbents (biomaterials, ion-imprinted materials, C_{60}–C_{70} and their derivatives, mesoporous materials, magnetic materials, and nanometer-sized materials) [Xiashi et al, 2010]. These new adsorbents can control analytical parameters such as selectivity, affinity and capacity [Poole, 2003].

Nowadays, a great number of publications are related to the applications of ionic liquids (ILs) in the field of analytical chemistry [Ping et al, 2010; Berthod et al, 2008]. Ionic liquid extraction has inspired the great interesting of examiners, including liquid-liquid extraction (LLE), liquid phase micro-extraction (LPME), stationary phase or the additives of mobile phase in GC/HPLC) ect. [Guor-Tzo et al, 2003; Jingfu et al, 2004; Antonio et al, 2009; Xiaojie et al, 2011]. Ionic liquid-modified silica particles (physical/ chemical modification) have already been developed as new sorbents to analyze metals [Xiaoqi et al, 2009] and organic compounds [Fei et al, 2009]. Tian et al. reported SPE-HPLC separation/analysis tanshinones using ionic liquid- modified silica sorbents [Minglei et al, 2009]. However, the determination of traditional Chinese medicine with IL-SiO_2 coupled to spectrometry seems to lack. In this work, separation/analysis of honokiol and magnolol was first conducted by IL-SiO_2 -fluorescence. The proposed method has been satisfactorily applied to the determination of honokiol and magnolol in Huoxiang Zhengqi Shui. In addition, the results showed that there was no significant difference between the proposed and the standard method of Pharmacopoeia of People's Republic of China [National Pharmacopoeia Commission, 2005].

Experimental

Apparatus and Reagents

The fluorescence measurements were performed on F-4500 spectrofluorimeter (Hitachi, Japan) equipped with a high-pressure xenon lamp and a quartz cell. The pH was measured on a pHS-25 pH-meter (Shanghai, China). Fourier transform infrared spectroscopy (FTIR, Tensor 27) (Bruker Company, Germany), Pyris-1 TGA (PE Company, USA), S-4800 field emission scanning electron microscope (FE-SEM) (Philips company, Dutch, 15KV, 0μA) were used for characterization.

Honokiol and magnolol standard were purchased from Chinese Control of Pharmaceutical and Biological Products. Sodium dodecyl sulfate (SDS), Silica gel (200–300 mesh), acetonitrile and ether were all purchased from Sinophar Chemical Regeant Co. Ltd. (China). N-methylimidazole, potassium hexafluorophosphate, 3-chloropropyltriethoxysilane was from Shanghai Crystal Pure Industrial Co. Ltd. (China). Huoxiang Zhengqi Shui was purchased from local market. Doubly distilled water (DDW) was used throughout.

Honokiol and magnolol stock solution of 100.0 $\mu g{\cdot}mL^{-1}$ were prepared. Working standard solutions were obtained by appropriate dilution of the stock standard solutions. The pH was controlled by buffer solutions (NaAc-HAc or NH_3- NH_4Cl).

Preparation and Characterization of Ionic Liquid-Modified Silica

1) Prepatation of Ionic Liquid-Modified Silica[31]

The commercial silica was activated by the following procedure to enhance the surface contents of silanol groups: 10.0 g silica was boiled with hydrochloric acid-water (1:1v/v) at 75°C for 10 h and immersed for 12h. The solid product was recovered by

filtration, washed with DDW to neutral, and dried at 90°C for 12h.

IL-silica was prepared as follows: (1) equal amount 0.2 mol of N-methylimidazole and 3-chloropropyltriethoxysilanen were mixed in a 500 mL round bottomed flask, and the mixture was refluxed with stirring for 48 h at 80°C. (2) after the reaction was cooled to room temperature, 36.8 g of KPF_6 dissolved in 300 mL acetonitrile was slowly added into the flask and the anion-exchange reaction was allowed to take place over the next 48 h. (3) 4.0 g of activated silica was mixed with silane-coupling agent attached with N-methylimidazolium ionic liquid and the mixture was refluxed with stirring for 24h. (4) the product was recovered by filtration and consecutively rinsed with ether (200 mL), acetonitrile (200 mL), and DDW (400 mL). The obtained ionic liquid-functionalized silica was dried at 80°C for 12 h. The main reaction schemes were shown in Fig. 2.

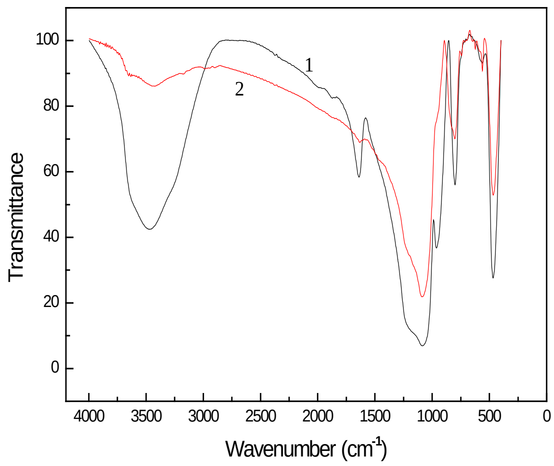


FIGURE 3 FTIR SPECTRA OF BARE SIO_2 (CURVE 1) AND IL-SIO_2 (CURVE 2)

2) *FTIR Analysis*

Fig. 3 shows the FTIR spectra of bare SiO_2 (curve 1) and IL-SiO_2 (curve 2). As it can be seen in curve 1 that there is a wide absorption peak at 3500 cm^{-1} from the vibration of Si–OH. In curve 2, the peak is changed significantly because there is less amount of free Si–OH on the surface of IL-SiO_2. These could indicate that ionic liquid has been grafted on the surface of silica.

3) *Surface Coverage Determination [Mohamed E et al, 2009]*

Thermal gravimetric analysis (TGA) as well as thermal desorption method was used to evaluate the surface coverage of IL-SiO_2. Pyris1-TGA was applied and commercial silica and ionic liquid-modified silica were heated at a rate of 10°C·min^{-1} from 50 to 600°C in nitrogen atmosphere. Thermogravimetric curves of SiO_2 and IL-SiO_2 are shown in Fig. 4. By comparison between curve 1 (SiO_2) and curve 2 (IL-SiO_2) in Fig. 4, it can be seen that there is an initial loss of weight at temperature below 100°C for all samples. This was attributed to the evaporation of water. In addition, curve 2 showed two very distinct thermal events. For the first one, 6.30% mass loss was observed in 100-320°C, which was attributed to the reduction of organic moieties on the surface. On the other hand, a mass loss of 5.35% in 320-570°C probably came from the ILs decomposition of IL-SiO_2. According to the mass loss in 100-570°C, the IL surface coverage of IL-SiO_2 was 0.410 mmol·g^{-1}.

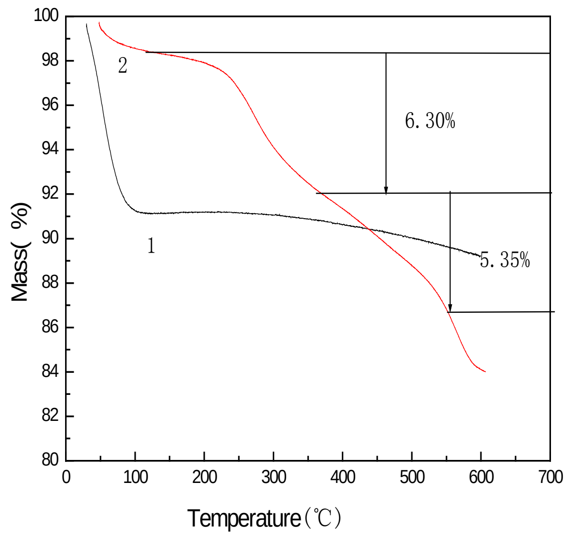


FIGURE 4 THERMOGRAVIMETRIC CURVES OBTAINED FOR (1) SIO2 AND (2) IL-SIO2

In thermal desorption method, 100±1mg of dry SiO_2 and IL-SiO_2 was weighed and put into a muffle furnace. The initial temperature was set at 25°C and gradually increased to 600°C in 0.5 h. The ignited silica was kept at this temperature for 2 h and then cooled down to 100°C inside the furnace. Finally the sample was transferred to desiccators and continually cooled to reach room temperature. The weight loss of hydrophobic ionic liquid was determined by comparing the sample masses before and after thermal desorption. The IL surface coverage of IL-SiO_2 was calculated as 0.424 mmol·g^{-1}.

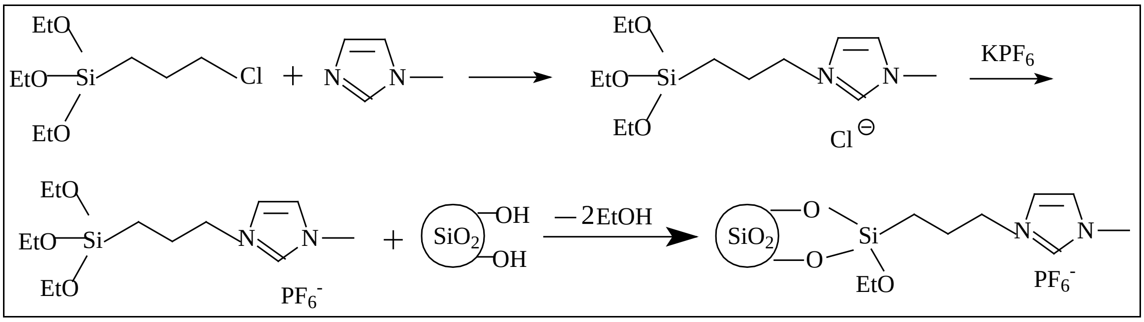
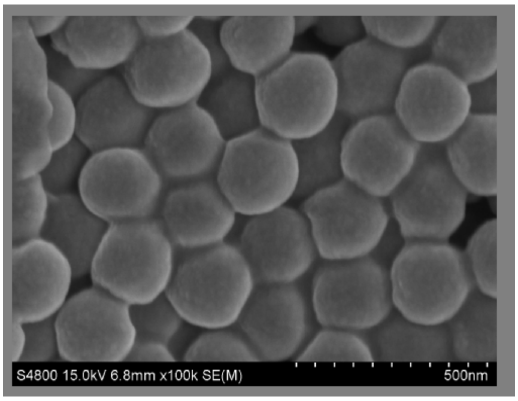


FIGURE 2 THE PREPARATION SCHEME OF IONIC LIQUID-MODIFIED SILICA

4) *Scanning Electron Microscope Of Il-SiO2*

The surface image of SiO_2 and IL-SiO_2 through the scanning electron microscopy was shown in Fig. 5. It could be seen clearly that IL-SiO_2 was successfully prepared.



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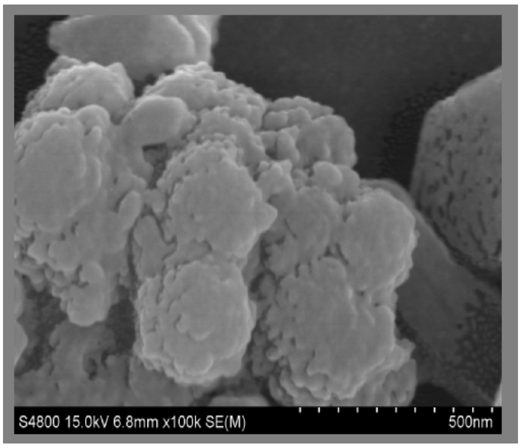

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FIGURE 5 THE SURFACE IMAGE OF SIO_2 (A) AND IL-SIO_2 (B)

Static Adsorption Procedure

In a 25.0 mL volumetric flask, the analytical solution containing appropriate amounts of honokiol and magnolol, SDS (0.36 mg·mL^{-1}, merely to honokiol) and pH 9.0 buffer solution were added. The mixed solution was diluted to the mark with distilled water, shaken thoroughly, and the fluorescence intensity (F_0) of honokiol and magnolol was measured at 353 nm, 412 nm upon excitation at 292 nm, 320 nm, respectively.

For static adsorption, in a centrifugal tube, 30.0 mg of IL-SiO_2 and 15.00mL above mentioned solution was added. The mixture was shaken on a timing multifunctional oscillator for 60 min and then centrifugal separation. Honokiol and magnolol in the water phase was detected by fluorospectrophotometry (F_1).

Elution Method

The elution behaviors of honokiol and magnolol from IL-SiO_2 were investigated with 2.5 mL of SDS solution (3%, m/v) and NaOH solution (0.2 M), respectively. The eluent was detected by fluorospectrophotometry (F_2). This enrichment factor (the volume ratio of adsorption solution and eluent) was 6.

The adsorption efficiency and elution efficiency were calculated as follows:

$$E_{adsorption}(\%) = \frac{C_0 - C_1}{C_0} \times 100\% \tag{1}$$

$$E_{elution}(\%) = \frac{C_2}{6C_0} \times 100\% \tag{2}$$

C_0 and C_1 were the concentration of analyte in aqueous phase before and after adsorption, respectively. C_2 was the concentration of analyte in eluent.

Hplc Analysis

The chromatography system consisted of LC-10AVP liquid chromatography equippment, L-7100 constant flow pump, SPD-10AVP oven, SCL-10AVP system controller and Class-VP chromatograph working station (Shimadzu, Japan). Chromatographic separations were performed on a commercial C_{18} (150 mm×4.6mm i.d., 5μm). The column was run with the mobile phase of acetonitrile: methanol: water =20:40:40 (v:v:v) at a flow rate of 1.0 mL·min^{-1} and monitored at 294nm. The injection volume was 20 μL. Each compound was dissolved in methanol.

Sample Preparation [National Pharmacopoeia Commission, 2005]

5.00mL Huoxiang Zhengqi Shui (Shuzhong Pharmaceutical Co.Ltd, China) and 2 drops of hydrochloric acid were mixed. The mixture was extracted with 3×10 mL chloroform. Integrated the extractant and evaporated at low temperature. Finally, the remains were dissolved with methanol to 10.0mL. Took 5.00 mL into a 10.0mL volumetric flask, diluted with methanol, filtrated with 0.45 μm organic membrane and analyzed as soon as possible.

Results and Discussion

Effect of pH

pH value plays a very important role in the SPE procedure. An appropriate pH value can not only improve the adsorption efficiency but also depress the interference of the matrix. According to the recommended procedure, the pH values of sample solutions were adjusted to the range of 6–11 to investigate the adsorption behaviors of honokiol and magnolol on sorbents (IL-SiO_2 and SiO_2). The results were shown in Fig. 6. It could be seen that the adsorption efficiencies of honokiol and magnolol with IL-SiO_2 as sorbents were much higher than with bare silica. Moreover the highest adsorption efficiency was got and kept unchanged between pH 7.0 and 10.0. The possible reason was the main existing form of analytes were various at different pH value (honokiol: pKa_1 =9.64, pKa_2 =10.71. magnolol: pKa_1=9.63, pKa_2=10.57). In this work, pH=9.0 was selected. In this condition honokiol and magnolol exists in neutral molecules, which have stronger hydrophobic and could be more easily adsorbated.

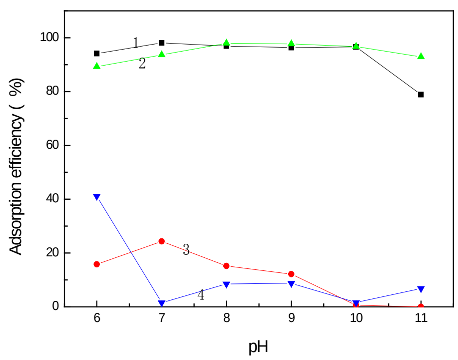

FIGURE 6 EFFECT OF PH ON THE ADSORPTION EFFICIENCIES OF HONOKIOL AND MAGNOLOL ON IL-SIO_2 AND SIO_2

Curve 1 and 2: the adsorption efficiency of honokiol and magnolol on IL-SiO_2; Curve 3 and 4: the adsorption efficiency of honokiol and magnolol on SiO_2

Select and Optimize Organized Molecular Assemblies

The organized molecular assemblies (surfactant, microemulsion, ionic liquid), may enhance the sensitivity of spectral analysis and change hydrophobic of the analytes so as to raise the adsorption efficiency. Table 1 showed that the adsorption behaviors were greatly affected by the type of organized molecular assemblies. For honokiol the adsorption efficiency was the highest in SDS system. While for magnolol, in water the adsorption efficiency was similar to that in β-CD and ionic liquid and relatively higher than that in CTAB and SDS.

Fig. 7 displayed the effect of SDS concentration on the honokiol adsorption efficiency. The adsorption efficiency reached a maximum when C_{SDS}=0.3~1.5 mg·mL^{-1}. But the adsorption efficiency was decreased when C_{SDS}> 1.5 mg·mL^{-1}. There were two reasons for this phenomenon (1): a special cooperative action may exist between SDS and ILs on the surface of sorbents. The hydrophobic of sorbents will be increased when they reached a specific proportion, which was helpful for the adsorption behavior. (2): the reason of adsorption efficiency decreased when C_{SDS}>1.5 mg·mL^{-1} was that honokiol in the solution was solubilizated into the micelle of SDS, which competed with the adsorption behavior on adsorbents. In this paper, 0.36 mg·mL^{-1} SDS was used for honokiol adsorption. For magnolol there was no need to use any organized molecular assemblies to improve the adsorption efficiency.

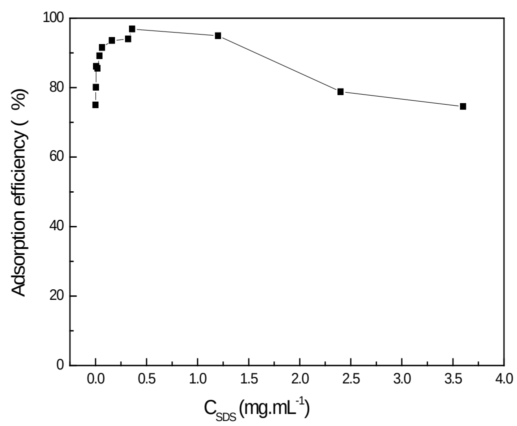

FIGURE 7 EFFECT OF SDS CONCENTRATION ON THE ADSORPTION EFFICIENCY OF HONOKIOL

Effect of Shaking Time

The sample adsorption time should be optimized to ensure quantitative retention. The adsorption kinetic

process of IL-SiO_2 at room temperature with pH 9.0 was studied. The results showed with the increase of adsorption time t (0.5–1.5 h), the percentages of honokiol and magnolol retained on IL-SiO_2 were increased. The adsorption efficiencies of honokiol and magnolol changed slightly when t was greater than 1.5 h. To shorten the analysis time, the shaking time was fixed at 1 h in the following experiments.

TABLE 1 EFFECT OF THE ORGANIZED MOLECULAR ASSEMBILIES ON THE ADSORPTION EFFICIENCIES OF HONOKIOL AND MAGNOLOL

The type of media (C=0.40 mg·mL^{-1})	Water	CTAB	SDS	β-CD	C_4minBr	C_{14}minBr
Adsorption efficiency of honokiol (%)	75.00	71.61	98.60	78.29	86.63	85.64
Adsorption efficiency of magnolol (%)	96.64	75.61	86.20	96.21	97.38	95.82

Effect of Temperature

In this experiment, the adsorption efficiencies of honokiol and magnolol at different temperatures (20~70°C) were studied. The results illustrated that both honokiol and magnolol can be quantitative adsorbed by IL-SiO_2 (>90%) at room temperature.

Effect of the Sample Volume

The amount of honokiol/magnolol was fixed at 10.0 μg and the volume of the sample solution was changed from 5.0 mL to 50.0 mL. The results were shown in Fig.8. It was observed that the adsorption efficiency was lesser than 90% when the volume of sample was over 25.0 mL. In this work, the sample volume of 15.0 mL was adopted.

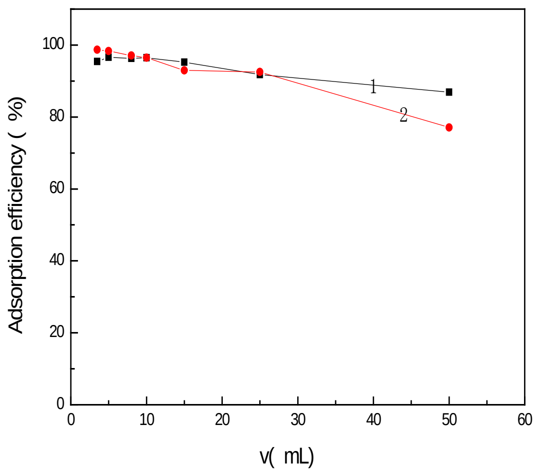

FIGURE 8 EFFECT OF SAMPLE VOLUME ON THE ADSORPTION EFFICIENCIES OF HONOKIOL AND MAGNOLOL

1: the adsorption efficiency of honokiol; 2: the adsorption efficiency of magnolol

Adsorption Capacity of IL-SiO_2

Adsorption capacity (the maximum adsorbed amount of analyte on 1.0 g sorbent) is an important factor to evaluate sorbent. The method used for capacity study was adopted from Xiashi et al. [Xiashi et al, 2009]. The amount of 0.030 g IL-SiO_2 was equilibrated with 15.00 mL solution in the concentration range of working curve. Fig. 9 shows that the adsorption capacity of IL-SiO_2 for honokiol and magnolol were 6.9 mg·g^{-1} and 6.0 mg·g^{-1}, respectively.

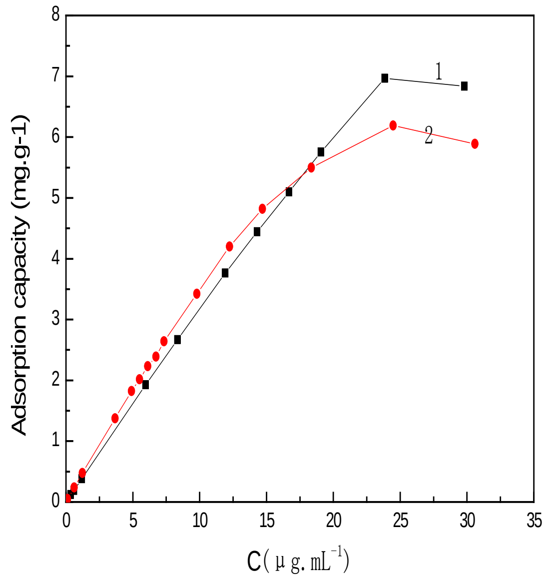

FIGURE 9 ADSORPTION CAPACITY

1: the adsorption capacity of honokiol; 2: the adsorption capacity of magnolol

Optimization of Elution Conditions

1) Selection of Eluent

The reuse of IL-SiO_2 was investigated with different elution (strong acid, strong base and the organized molecular assembilies) in this experiment. The results are listed in Table 2. Both strong acid and strong base could not elute the honokiol, which was in accord with "Effect of pH". However, it was discovered that the organized molecular assembilies could elute the honokiol. It confirmed again that there was competition between the solubilization behavior of honokiol into SDS micelle and the adsorption of honokiol on IL-SiO_2.

Meanwhile, certain concentration of NaOH solution could quantitatively elute the magnolol on IL-SiO_2. So, SDS was investigated as eluent for honokiol and NaOH for magnolol.

TABLE 2 EFFECT OF TYPES OF ELUENT ON THE ELUTION EFFICIENCY

Types of eluent (3.00 mL, C=3% (m/v))	Elution efficiency (%)		Types of eluent (3.00 mL,C=3% (m/v))	Elution efficiency (%)	
	Honokiol	Magnolol		Honokiol	Magnolol
HNO_3 (2.0 mol/L)	1.5	0.1	SDS	70.2	6.6
NaOH (0.2 mol/L)	5.5	82.3	β-CD	12.3	9.5
Triton-100	50	13.9	C_4minBr	5.2	0.7
CTAB	63.7	34.3	C_{14}minBr	10.1	5.7

2) Optimization of Concentration and Volume of Eluent

Desorption of Honokiol

SDS selected to elute the honokiol could quantitatively elute honokiol (70%) when C_{SDS} is greater than 30 mg·mL^{-1} (Fig.10). The effects of shaking time and the volume of elution on the elution efficiency of honokiol were also studied. The elution efficiency of honokiol showed scarcely variation when t is longer than 0.5 h and the influence of elution volume is very small. So optimum volume of SDS solution (3.0%, m/v) chosen for this work was 2.5 mL, with pre-concentration factor of 6.

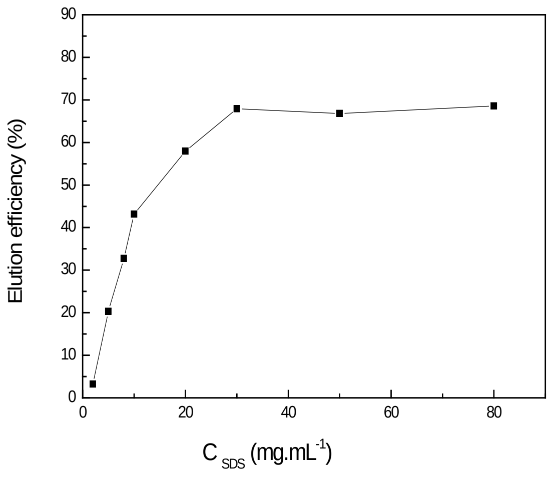

FIGURE 10 EFFECT OF SDS CONCENTRATION ON THE ELUTION EFFICIENCY OF HONOKIOL

Desorption of Magnolol

The elution efficiency of magnolol with 0.02-0.80 M of NaOH solution was investigated (Fig.11). The elution efficiency of magnolol kept about 80% when C_{NaOH}=0.10-0.50 M. In addition, it was found that the sorbents could be dissolved when the shaking time was longer than 5 h or C_{NaOH} >2.0 mol·L^{-1}, meaning that the sorbents is unstable in strong basic circumstances. So the elution must be analyzed as soon as possible.

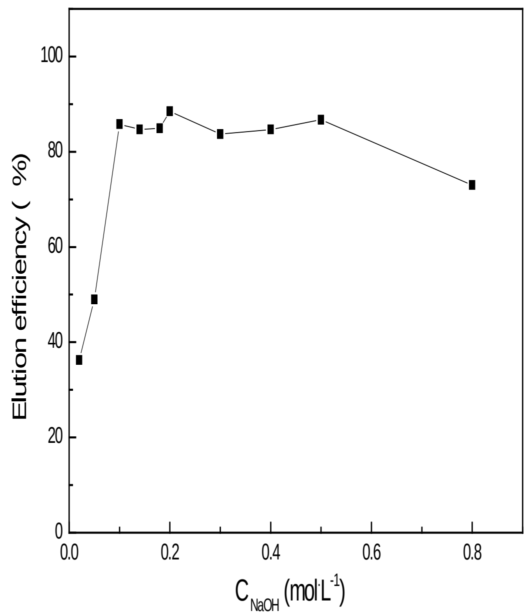

FIGURE 11 EFFECT OF NAOH CONCENTRATION ON THE ELUTION EFFICIENCY OF MAGNOLOL

Reversibility and Reproducibility of IL-SiO2

IL-SiO_2 was reproduced 10 times for honokiol (only 2 times for magnolol) as "Static Adsorption Procedure" and the retention efficiency kept stable. It was demonstrated that IL-SiO_2 exhibited good reversibility and reproducibility for honokiol and the adsorbent was unstable in strong basic circumstances.

Interferences

Determination of honokiol (1.0μg·mL^{-1}) and magnolol (0.48 μg·mL^{-1}) in the presence of foreign substances were investigated. With a relative error less than ±5%, the tolerance limits for various foreign substances are listed in Table 3 (tolerance ratio in mass). As indicated in table 3, there is little interference from the possible existing substances.

TABLE 3 EFFECT OF FOREIGN SUBSTANCES ON DETERMINATION OF HONOKIOL AND MAGNOLOL

Substances	Tolerance ratio (honokiol)	Tolerance ratio (magnolol)	Substances	Tolerance ratio (honokiol)	Tolerance ratio (magnolol)
Gastrodin	50	300	Mo^{6+}	500	5000
Tanshinone Ⅱ	5	20	Zn^{2+}	100	5000
Sodium tanshinone	3.5	5	Na^{+}	100	5000
Ferulaic acid	3.5	3	Cd^{2+}	50	20
Baicalin	2.5	3	Cu^{2+}	5	500
Andrographolide	2.5	20	Pb^{2+}	3	3
Magnolol	3	_	Fe^{3+}	2	20
Honokiol	_	20	Al^{3+}	2	500

TABLE 4 THE PERFORMANCE CHARACTERISTIC OF PROPOSED METHOD

Substance	The linear equation	Correlation (r)	The linear range (μg·mL^{-1})	R.S.D(%)	LOD(ng·mL^{-1})
honokiol	y=927.19 x+352.98	0.9913	0.015-7.60	4.98	2.6
magnolol	y=1742.31 x+192.49	0.9963	0.008-5.00	4.00	1.3

TABLE 5 DETERMINATION RESULTS OF HUOXIANG ZHENGQI SHUI SAMPLES (MEAN±S.D., N = 3)

Substance	The proposed method	The standard method	RSD
Honokiol (μg·mL^{-1})	273.48±0.04	275.75±0.02	0.82%
Magnolol (μg·mL^{-1})	250.12±0.03	243.66±0.02	2.58%

Note: the original solution was diluted 500 times

TABLE 6 DETERMINATION RESULTS OF TAP WATER AND RECOVERY (MEAN±S.D., N = 3)

Tap water	Honokiol (μg·mL^{-1})		Recovery (%)	Magnolol (μg·mL^{-1})		Recovery (%)
	Added	Found		Added	Found	
1	0	0.052	/	0	0.104	/
2	0.880	0.856±0.012	91.8	0.672	0.722±0.010	93.0
3	1.760	1.972±0.004	108.8	1.344	1.509±0.002	104.2
4	2.640	2.689±0.008	99.9	2.016	2.115±0.012	99.8
5	3.520	3.445±0.002	96.4	2.688	2.718±0.012	97.3

TABLE 7 COMPARISON WITH OTHER PREVIOUSLY REPORTED METHODS

Sample	Method	Linear range [a]	LOD	Reference
Plasma	HPLC	A:20—200 ng·mL^{-1} B: 40—400 ng·mL^{-1}	13 ng·mL^{-1} 25 ng·mL^{-1}	[Xin-an et al, 2003]
Cortex magnoliae officinalis	CE- electrochemical detection	A:0.001-1.0 mM B:0.001-1.0 mM	0.38 μM 0.51 μM	[Chen G. et al, 2006]
Houpu	Mesoporous silica-based electrochemical sensor	A:2.0-100 μg·L^{-1} B:20-300 μg·L^{-1}	0.5 μg·L^{-1} 10 μg·L^{-1}	[Jun et al, 2009]
Xiangsha Pingwei, Huoxiang Zhengqi Shui, Insurance agent pill	UV	A:2.66-79.8 μg·mL^{-1} B:2.66-79.8 μg·mL^{-1}	——[b] ——	[Bao et al,2004]
Rabbit plasma	Micelle-stabilized fluorimetry	A:0.4-6 μg·mL^{-1} B:0.2-6 μg·mL^{-1}	0.02 μg·mL^{-1} 0.002 μg·mL^{-1}	[Bai et al, 1999]
Huoxiangzhengqi oral solution	SPE-HPLC	A:2.60-208 μg·mL^{-1} B:5.45-436 μg·mL^{-1}	—— ——	[Zhang et al, 2009]]
Huoxiang Zhengqi Shui	SPE- fluorimetry	A:0.015-7.60 μg·mL^{-1} B:0.008-5.00 μg·mL^{-1}	2.6 ng·mL^{-1} 1.3 ng·mL^{-1}	This work

a: A was Honokiol and B was Magnolol; b: "-'means there were no specific data in the article

Analytical Parameters

Under optimal conditions, the calibration graph for the determination of honokiol and magnolol was obtained by plotting fluorescence intensity versus concentration. All analytical parameters are listed in Table 4.

Sample Analysis

The accuracy of the proposed method was examined through determining the amounts of honokiol and magnolol in different samples, i.e. honokiol and magnolol reference substances, Huoxiang Zhengqi Shui and tap water by standard curve method and standard adding method, respectively. Results are listed in Table 5, 6. The t-test method was used to do a significant difference test for proposed method and the standard method (regulated by Pharmacopoeia of People's Republic of China), and it was found that the

data of both methods did not have significant difference ($t=1.03$, $t<t_{0.10, 9}$). Compared with other reported methods (Table 7), the advantages of the present work are mainly focused on the relatively high sensitivity, low detection limit or wide linear range.

Conclusion

In the paper, novel ionic liquid-modified silica sorbents (IL-SiO_2) were prepared by the surface chemical modification on commercial silica and characterized by FT-IR, TGA and SEM analysis technology. Subsequently, the separation/analysis of honokiol and magnolol was first proposed by IL-SiO_2-fluorescence. Under the optimal conditions, the adsorption efficiencies of honokiol and magnolol on IL-SiO_2 were much higher than that of bare silica. The developed method has been successfully applied to the determination of honokiol and magnolol in Huoxiang Zhengqi Shui.

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